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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
JJ-10 072WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/00628	13/07/1999	13/07/1998
International Patent Classification (IPC) or national classification and IPC A61L9/01		
Applicant LIFE SCIENCE TGO, SRL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 12 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/02/2000	13.10.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Semino, D Telephone No. +49 89 2399 7324

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/00628

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-5,7,8,10-15, 19-21	as originally filed			
6,9,16-18	as received on	28/07/2000	with letter of	28/07/2000

Claims, No.:

1-35	as received on	28/07/2000	with letter of	28/07/2000

Drawings, sheets:

1/5-5/5	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-35
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-35
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-35
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I
Basis of the report

1. The opinion has been established as if the following amendment had not been made, since it has been considered to go beyond the disclosure as filed (Article 34(2)(b) PCT):

a. the addition on p. 17, l. 14-15 of the words 'of Example 1'.

Re Item V
Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. **Pertinence of the cited prior art**

1.1 Document D1 (CS-A-246119) relates (cf. abstract from Chemical Abstracts) to a method for removing biological impurities from textile material using an activated culture of Scotobacteria.

1.2 Document D2 (US-A-4925707) discloses (cf. claim 1) a process of imparting stain resistance to an installed nylon carpet by applying a stain-blocker selected from sulphonate phenol formaldehyde condensate polymers, sulfonated naphtol formaldehyde condensate polymers, and hydrolysed vinyl aromatic maleic anhydride polymers. Fluorochemical soil-resist agents may be added (cf. claim 19).

1.3 Document D3 (WO-A-9619611) discloses (cf. claim 1 and abstract) a method of producing wool with improved properties comprising the steps of treating wool in a plasma treatment process or in the Delhey process (cf. also p. 11) and subjecting the wool to a treatment with a proteolytic enzyme. The enzyme can be produced by bacteria (e.g from Bacillus strain, cf. p. 14-15).

1.4 Document D4 (US-A-4680212) discloses (cf. claim 1) a stain resistant nylon fibre with a coating on the surface comprising one or more stain blockers. Preferred stain blockers are polymeric condensation product (cf. claim 10); one or more fluorochemicals may be added (cf. claim 9).

1.5 Document D5 (JP-A-9028377) relates (cf. WPI abstract) to a microbe-impregnated water-absorbing polymer to be used as fertiliser, deodorant and waste water treating agent.

2. **Conclusions**

2.1 The method in claim 1 differs from the method in D1, which can be considered the closest state of the art, for the fact that the bacteria are dormant and are capable of becoming active when the fibres are exposed to organic material so as to digest the organic material and control odours.
The problem to be solved is how to provide a method as in D1 in which the effects of removal of biological material and odour control are long lasting.
There are no hints in the prior art to solve the problem as in the method in claim 1, which is therefore novel and inventive (Article 33(2) and (3) PCT).

2.2 The same as in paragraph 2.1 applies *mutatis mutandis* to the composition of claim 13 and to the carpet of claim 24.

Re Item VII
Certain defects in the international application

0. The following items of information are merely for the sake of expediency in case of any further regional examination before the EPO.

1. The expression 'incorporated herein by reference' (p. 2, l. 22-23) is not in conformity with Rule 1(a)(ii) and (iii) (see also the PCT Guidelines, II-4.17). The expression should be cancelled, in so far the description should be sufficient *per se*.

2. The units of weight on page 9, l. 15 ('oz') are not additionally expressed in terms of the units stipulated by Rule 10.1(a) PCT. Moreover, it is not apparent whether the applicants in this context wish to refer to a total weight or to a weight per unit surface.

3. It is not apparent whether Example 3 is an example of the invention, since no

treatment of fibres is accomplished.

Re Item VIII
Certain observations on the international application

1. The statement on p. 4, l. 26-29 and the wording of claims prompt to interpret that the fact that the bacteria are **dormant** is an essential feature of the invention. This is further confirmed by the conclusions under Item V.
However, this is in contrast with the teaching on p. 9, l. 23 ('Preferably') and on p. 11, l. 4-9 and 19-20, resulting therefore in lack of clarity (Article 6 PCT).

2. It is not clear what is meant by the wording of claims 10, 21 and 33 (Article 6 PCT). A clear basis can be found on p. 10, l. 29-33 ('the preparation is applied in an amount so as to result...'). Moreover, the word 'treat' in claim 11 should read 'treatment'.

3. The vague and imprecise statement in the description on page 21, last paragraph ('spirit of the invention') implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

Figure 3 is a graph illustrating the germination and growth of the bacterial spore blend in nylon carpet containing various organic soils; and

Figure 4 is a graph illustrating the germination and growth of the bacterial spore blend on carpet containing a combination of fox urine and dog feces.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed in one aspect to a method of controlling odor associated with deposits, particularly spills, of organic material which can cause odors on carpet or other fibrous materials. The present invention is also directed to the compositions useful for preparing carpet or other fibrous material to make them capable of controlling odor as well as to the carpet or other fibrous material so prepared. In addition to controlling odor, the compositions may also aid in reducing the staining effects of organic material.

Many bacterial genera are known to produce enzymes which are capable of breaking down organic material. Such bacteria are particularly useful where the organic material, if allowed to remain, will give rise to malodors. Several such bacterial genera such as Bacillus, Lactobacillus, Enterobacter, Streptococcus, Nitrosomonas, Nitrobacter, Pseudomonas, Alcaligens and Klebsiella amongst others are known for use in such applications, with Bacillus and Lactobacillus sp. being the most prevalent in use in various applications. Strains of bacteria from any of the above noted genera are useful in practicing the present invention. Preferably, the bacterial preparation for use in the present invention is one or more strains of Bacillus or Lactobacillus. More preferably, the strains of bacteria for use in the present invention are selected from Bacillus licheniformis, Bacillus pasteurii, Bacillus laevolacticus and Bacillus amyloliquefaciens. Each of these species have characteristics which make them most effective against particular types of organic materials.

the bacteria will undergo rapid growth and consume the odor causing material. The factors which can affect the number of bacteria to be used relate in most part to the nature of the carpet material. Such factors include the nature of the fiber in terms of the material, e.g. nylon or polypropylene and the like, the characteristics of the yarn in the terms of the denier and number of filaments and the characteristics of the fiber in terms of the number of yarns and the twist. These factors relate to the nature of the carpet in terms of the weight (oz) and height of the pile. All of these factors will affect the amount of exposed surface of the fibers which might be covered by the bacterial preparation. For most applications on carpet, between about 10^6 and 10^8 cells per gram of carpet fiber having a weight between about 20oz and 40oz is most effective with 10^7 cells per gram of carpet fiber being most preferred.

The preparations may be provided as a simple aqueous preparation of a suspension of the Bacillus species in a suitable aqueous carrier, such as in distilled water, tap water, a saline solution or other such aqueous solutions. Preferably, the aqueous composition comprises the odor controlling dormant bacterial strain or strains and an effective amount of a stain blocker. The stain blocker is preferably selected from the group consisting of sulfonated phenol formaldehyde condensate polymer, a sulfonated naphthol formaldehyde condensate polymer, a hydrolyzed vinyl aromatic maleic anhydride polymer or combinations thereof. The aqueous composition may also include one or more fluorochemicals typically utilized for carpet treatment, either on their own or in combination with the stain blocker. Examples of such fluorochemicals include products sold under the trademarks STAINMASTER, STAINMASTER with TEFLON, and ZONYL by DuPont and SCOTCHGARD by 3M.

medical cotton rolls used as wicks to increase the surface area of the caustic solution. Each reactor was provided with sufficient carpet material to yield 5 grams of carpet fiber. A plate count broth prepared by mixing 17g Difco Plate Count Broth, 0.073g KH_2PO_4, 0.114g K_2HPO_4 per liter of distilled water and the pH adjusted to 7 was added to the reactor and the reactors autoclaved to sterilize them. The reactors were allowed to cool and 0.5 ml of the bacterial suspension utilized in Example 1 containing 10^8 spores per ml were added to the test reactors. The same volume of distilled water was added to the control reactors. The reactors were capped without the caustic traps and rolled and swirled to ensure that the water and bacterial preparations were mixed well with the organic materials and to permit the carpet to absorb the liquid. The caustic traps were then inserted into the reactors and the reactors hooked up to the respirometer systems. The reactors were incubated in a water temperature bath maintained at 23 °C using an automatic temperature controller. The oxygen uptake by any bacteria growing in the reactors was monitored continuously and reported at 2 hour intervals.

As illustrated in Figure 2, carpet fiber which had not been inoculated with the bacterial spore blend demonstrated only a very slight increase in oxygen uptake after about 24 hours of incubation. The oxygen uptake did not increase above this level up to 60 hours post-inoculation. These results indicate minimal bacterial growth in the control carpet sample. In contrast, the carpet fiber inoculated with the bacterial spore blend showed an increase in oxygen uptake starting 22 to 24 hours after inoculation. This increase in oxygen uptake continued up to the end of the test at 60 hours post-incubation with the oxygen intake increasing in a steady linear fashion with no leveling off of the uptake seen during the 60 hours of the test. These results indicate that the dormant bacteria are capable of germinating to

become active and undergo growth in response to exposure to a suitable food source.

Example 3

To confirm that the bacterial spore blend utilized in the present invention could grow on various organic soils, plates containing materials representative of common household or soil causing organic based materials were inoculated with the bacterial spore blend. The organic based materials utilized were chocolate syrup, tomato sauce, milk, dog feces and fox urine. The growth on these soils was compared to a standard plate count broth utilized for counting colony forming units. The plates were inoculated with dilutions of the bacterial spore blend to give between about 300 and 400 spores per plate and incubated at 37°C and 50% humidity. At two days and four days post inoculation, the colony forming units (CFU) were counted and the CFU's per ml of the innoculum were calculated. After two days, the bacterial preparations were growing well on the tomato sauce, chocolate syrup and dog feces, with growth almost at the level of the standard plate count broth. A minimal increase in growth on the autoclaved milk or fox urine was observed after two days, although there was some growth. After four days, the growth on all five materials was comparable, being only slightly less than the growth on the plate count broth. These results indicate that the bacterial spore blend can grow well on common organic soil, such as chocolate syrup, tomato sauce, dog feces and fox urine.

Example 4

The bacterial spore blend was tested using respirometric studies as set out in Example 2 above to confirm that it could utilize pet waste for growth in carpets. Samples of the carpet fiber were examined for oxygen uptake using a standard respirometric study conducted using a Challenge AER100 respirometer with all samples incubated under controlled temperature conditions.

The treatment reactors were 500 ml bottles. The CO_2 adsorption trap inserts contained 5 ml of 30% KOH (w/v) with alizarin yellow pH indicator. The sterilized traps were filled with the KOH caustic solution then inserted into the sterilized reactors using aseptic techniques. The CO_2 traps also contained sterilized medical cotton rolls used as wicks to increase the surface area of the caustic solution. Each reactor was provided with sufficient carpet material to yield 5 grams of carpet fiber. The organic material (i.e. dog feces, fox urine, plate count broth, etc.) was added to the reactor and the reactors autoclaved to sterilize them. The reactors were allowed to cool and 0.5 ml of the bacterial suspension containing 10^8 spores per ml were added to the test reactors. The same volume of distilled water was added to the control reactors. The reactors were capped without the caustic traps and rolled and swirled to ensure that the water and bacterial preparations were mixed well with the organic materials and to permit the carpet to absorb the liquid. The caustic traps were then inserted into the reactors and the reactors hooked up to the respirometer systems. The reactors were incubated in a water temperature bath maintained at 23 °C using an automatic temperature controller. The oxygen uptake in the reactors was monitored continuously and reported at 2 hour intervals.

The carpet sample in the control reactor with no innoculum did not have any significant increase in oxygen uptake over the 96 hours of the test. The carpet samples which had been inoculated with the bacterial spore blend started showing an increase in oxygen uptake after 32 hours post-inoculation. This increase in oxygen uptake continued to the end of the test in a linear fashion with no plateauing of the oxygen uptake observed up to 96 hours post-inoculation. This clearly shows that the bacterial spore blend associated with the carpet can become activated and undergo growth when exposed to a common organic spill material.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method for controlling odor associated with deposits of organic material which can cause odors on carpets or other fibrous material, the method comprising applying to the carpet or other fibrous material or to the fibers used in the manufacture of the carpet or other fibrous material, a preparation of dormant bacteria, which when activated are effective to control odors, the dormant bacterial preparation being allowed to become associated with the carpet or other fibrous material such that when the carpet or other fibrous material is exposed to organic material which can cause odors, the bacteria are capable of becoming active and digesting the organic material.

2. A method as claimed in claim 1 wherein the dormant bacteria are sporulated forms of one or more strains selected from the bacterial genera Bacillus.

3. A method as claimed in claim 1 wherein the dormant bacteria are sporulated forms of one or more strains selected from the group of bacterial species consisting essentially of Bacillus licheniformis, Bacillus pasteurii, Bacillus laevolacticus and Bacillus amyloliquefaciens.

4. A method as claimed in claim 3 wherein the dormant bacteria are applied to the carpet at a concentration of between about 10^6 and about 10^8 cells per gram of carpet fiber.

5. A method as claimed in claim 4 wherein the dormant bacteria are applied to the carpet at a concentration of about 10^7 cells per gram of carpet fiber.

6. A method as claimed in claim 3 wherein the dormant bacterial preparation comprises:

	% of total bacteria	
Species	Range	Preferred
Bacillus licheniformis	20-60	40
Bacillus pasteurii	10-30	20
Bacillus laevolacticus	10-30	20
Bacillus amyloliquefaciens	10-30	20

7. A method as claimed in claim 3 wherein the bacterial preparation includes one or more stain-blocking chemicals.

8. A method as claimed in claim 7 wherein the one or more stain-blocking chemicals are selected from the group consisting of sulfonated phenol formaldehyde condensate polymer, sulfonated naphthol formaldehyde condensate polymer, and hydrolyzed vinyl aromatic maleic anhydride polymer.

9. A method as claimed in claim 8 wherein the preparation contains an amount of the stain blocker to result in a treat rate of the carpet of about 0.1 wt% to about 20 wt% based upon the weight of the carpet fiber.

10. A method as claimed in claim 9 wherein the treat rate is from about 0.25 wt% to about 20 wt%.

11. A method as claimed in claim 8 wherein the bacterial preparation further includes one or more anti-soil fluorochemicals.

12. An aqueous odor controlling bacterial composition for treating carpet or other fibrous material to impart odor control to the carpet or other fibrous material, the

composition comprising one or more stain-blocker chemicals and an effective amount of odor controlling bacteria.

13. An aqueous odor controlling bacterial composition as claimed in claim 12 wherein the bacteria are one or more strains selected from the group of bacterial genera consisting of Bacillus, Enterobacter, Streptococcus, Nitrosomonas, Nitrobacter, Pseudomonas, Alcaligens and Klebsiella.

14. An aqueous odor controlling bacterial composition as claimed in claim 13 wherein the bacteria are one or more strains selected from the group of bacterial species consisting essentially of Bacillus licheniformis, Bacillus pasteurii, Bacillus laevolacticus and Bacillus amyloliquefaciens.

15. An aqueous odor controlling bacterial composition as claimed in claim 14 wherein the bacteria are applied to the carpet or other fibrous material at a concentration of between about 10^6 and about 10^8 cells per gram of carpet fiber.

16. An aqueous odor controlling bacterial composition as claimed in claim 15 wherein the dormant bacteria are applied to the carpet or other fibrous material at a concentration of about 10^7 cells per gram of carpet fiber.

17. An aqueous odor controlling bacterial composition as claimed in claim 14 wherein the bacterial preparation comprises:

	% of total bacteria	
Species	Range	Preferred
Bacillus licheniformis	20-60	40
Bacillus pasteurii	10-30	20
Bacillus laevolacticus	10-30	20
Bacillus amyloliquefaciens	10-30	20

18. An aqueous odor controlling bacterial composition as claimed in claim 14 wherein the one or more stain-blocking chemicals are selected from the group consisting of sulfonated phenol formaldehyde condensate polymer, sulfonated naphthol formaldehyde condensate polymer, and hydrolyzed vinyl aromatic maleic anhydride polymer.

19. An aqueous odor controlling bacterial composition as claimed in claim 18 wherein the preparation contains an amount of the stain blocker to result in a treat rate of the carpet of about 0.1 wt% to about 20 wt% based upon the weight of the carpet fiber.

20. An aqueous odor controlling bacterial composition as claimed in claim 19 wherein the treat rate is from about 0.25 wt% to about 20 wt%.

21. An aqueous odor controlling bacterial composition as claimed in claim 20 wherein the bacterial composition further includes one or more anti-soil fluorochemicals.

22. A carpet capable of controlling odor associated with deposits of organic material which can cause odors on the carpet, the carpet comprising fibers tufted through a primary backing, the fibers having associated therewith a preparation of dormant bacteria, which when activated are effective to control odors, such that when the carpet is exposed to organic material which can cause odors, the bacteria are capable of becoming active and digesting the organic material.

23. A carpet as claimed in claim 23 wherein the bacteria are one or more strains selected from the group of bacterial genera Bacillus.

24. A carpet as claimed in claim 22 wherein the bacteria are one or more strains selected from the group of bacterial species consisting essentially of Bacillus

licheniformis, Bacillus pasteurii, Bacillus laevolacticus and Bacillus amyloliquefaciens.

25. A carpet as claimed in claim 24 wherein the dormant bacteria are applied to the carpet at a concentration of between about 10^6 and about 10^8 cells per gram of carpet fiber.

26. A carpet as claimed in claim 25 wherein the dormant bacteria are applied to the carpet at a concentration of about 10^7 cells per gram of carpet fiber.

27. A carpet as claimed in claim 24 wherein the dormant bacterial preparation comprises:

	% of total bacteria	
Species	Range	Preferred
Bacillus licheniformis	20-60	40
Bacillus pasteurii	10-30	20
Bacillus laevolacticus	10-30	20
Bacillus amyloliquefaciens	10-30	20

28. A carpet as claimed in claim 24 wherein the carpet has also been treated with one or more stain-blocking chemicals.

29. A carpet as claimed in claim 28 wherein the one or more stain-blocking chemicals are selected from the group consisting of sulfonated phenol formaldehyde condensate polymer, sulfonated naphthol formaldehyde condensate polymer, and hydrolyzed vinyl aromatic maleic anhydride polymer.

30. A carpet as claimed in claim 29 wherein the preparation contains an amount of the stain blocker to result in a treat rate of the carpet of about 0.1 wt% to about 20 wt% based upon the weight of the carpet fiber.

31. A carpet as claimed in claim 30 wherein the treat rate is from about 0.25 wt% to about 20 wt%.

32. A carpet as claimed in claim 29 wherein the carpet has also been treated with one or more anti-soil fluorochemicals.